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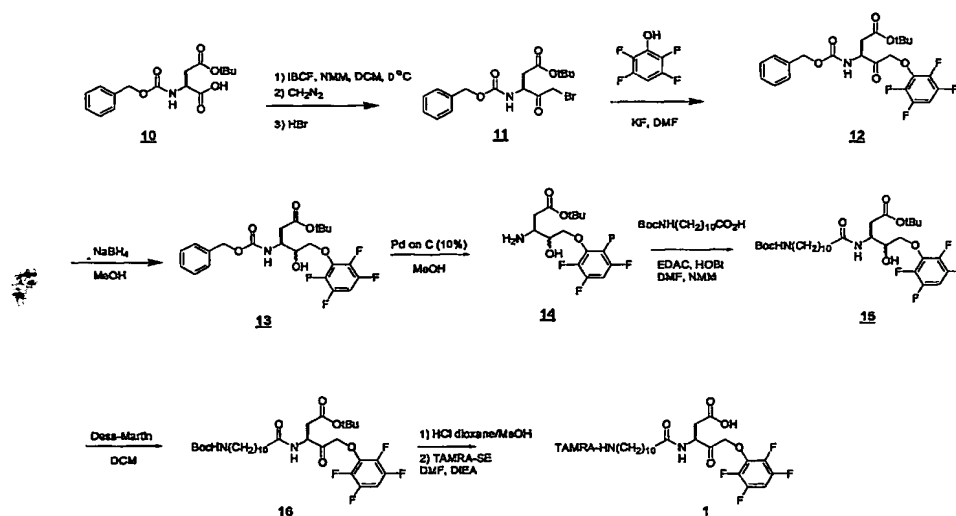
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(54) Title: **ACTIVITY-BASED PROBES, AND METHODS OF THEIR PREPARATION AND USE**



(57) Abstract: The present invention provides compositions and methods for assessing profiles of catalytically active enzymes in compositions containing a plurality of proteins. In preferred embodiments, the enzyme is a hydrolase, most preferably a cysteine protease. The methods described herein use activity based probes ("ABPs") that have an affinity moiety for directing the binding of the ABP to one or more catalytically active target enzymes, a reactive group for forming a covalent bond at an active site of the target enzyme(s), and a TAG (e.g., a detectable label, preferably a fluorophore). One or more ABPs may be combined with a protein-containing sample under conditions for binding and reaction of the ABP(s) with target enzyme(s) that are present in the sample. The resulting products may then be used to assess the active enzyme profile of the sample, and can be correlated to the presence, amount, or activity of one or more target enzyme(s) present in the original complex protein mixture.



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